Evaluation of Testicular Size and Function in 1–3-Year-Old Stallions

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Considerable variation exists in the level of spermatogenic efficiency present in similar sized testes of stallions ≤ 3 years of age. Results of 53 breeding soundness examinations of 3-year-old stallions composed of 8 breeds were compiled and demonstrated that scrotal width was only moderately correlated with parameters of semen quality in first or second ejaculates. Equine practitioners should be cautious about making predictions for mare books (number to be bred) without first examining semen quality. Authors’ addresses: College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475 (Blanchard); Nandi Veterinary Associates, 3244 W. Sieling Rd., New Freedom, PA 17340 (Johnson, Brinsko, Varner, Rigby, and Hurtgen). © 2001 AAEP.

1. Introduction
The equine practitioner is increasingly requested to evaluate size and normalcy of testes in young stallions for prepurchase examination, first year fertility insurance, or as part of a “breeding soundness” examination. The scientific literature is replete with references on testicular size and function in adult stallions (> 3 years of age), but far fewer references exist for comparison of testicular parameters in stallions ≤ 3 years of age. Evaluation of testes from stallions aged 1–5 years of age revealed that stallions reach adult values for spermatogenic efficiency (i.e., daily sperm production per gram [DSP/g] of testis) at 3 years of age, and sperm production per testis and per horse at 4 years of age. However, these investigators noted that considerable variation in these parameters existed among horses within same age groups. Since establishment of spermatogenesis in pubertal stallions has been shown to be more closely related to size of the testes than the age of the horse, we re-examined the relationships between spermatogenic efficiency (potential DSP/g of testis), germ cell losses during meiosis and spermiogenesis, and testis size in 20 sexually immature stallions (1–3 years of age) (Study 1). Additionally, to provide further information on testes size and semen quality in sexually immature stallions, we reviewed results from 53 breeding soundness examinations of 3-year-olds composed of 8 breeds (Study 2).

2. Materials and Methods
Study 1
Testes were obtained from 20 1–3-yr-old stallions during the months of May to July (breeding season; n = 10) and November to January (nonbreeding...
season; n = 10) from a commercial abbatoir. Ages of horses were determined by dental characteristics. Horses were combined into three age groups as follows: 1–1.5 yr = age 1 (n = 10); 2–2.5 yr = age 2 (n = 6); 3 yr = age 3 (n = 4). Testes were weighed and prepared for histomorphometric assessment, and the types and numbers of germ cells were determined as previously described. Potential DSP/g of testis during spermatogenesis (for early primary spermatocytes, late primary spermatocytes, round spermatids, and elongated spermatids) was estimated after correction of each germ cell type for its respective lifespan. Declines (i.e., percentage losses) in potential DSP/g of testis during spermatogenesis were calculated as previously described. Data were analyzed by two-way analysis of variance to detect age, season, and age by season differences. Correlation coefficients and linear regression analyses were performed to characterize the relationship between testis size, potential DSP/g, and percentage decline in potential DSP/g during meiosis and spermiogenesis.

Study 2
For Study 2, breeding soundness records of 53 3-year-old stallions of 8 breeds (Standardbred, Thoroughbred, Quarter Horse, Arabian, Warmblood, Paint, Appaloosa, Peruvian Paso) were used. Total scrotal width and semen quality in ejaculates (one ejaculate, or two ejaculates collected one hr apart) were examined. Evaluation of semen quality included total sperm number, percentage progressively motile sperm, total number of progressively motile sperm, percentage morphologically normal sperm, and total number of morphologically normal sperm. Data were analyzed by one-way analysis of variance to detect breed effects. Simple correlation coefficients were calculated to assess the relationship between scrotal width and parameters of semen quality.

3. Results
Study 1
No age, season, or age-by-season effects were detected for testis weight (p > 0.10). No age, season, or age-by-season effects were detected for potential DSP/g or for losses in potential DSP/g during meiosis (p > 0.10). Significant age, but no season or age-by-season (p > 0.10), effects occurred for potential DSP/g (p = 0.02) (Fig. 1) and for decline in potential DSP/g (p = 0.01) during spermiogenesis (Fig. 2). Testis weight was positively correlated with potential DSP/g based on enumeration of late primary spermatocytes (r = 0.43, p = 0.06), round spermatids (r = 0.74, p = 0.0003), and elongated spermatids (r = 0.81, p = 0.0001), and was negatively correlated with losses in potential DSP/g during meiosis (r = −0.67, p = 0.002) and spermiogenesis (r = −0.53, p = 0.04). Regression analysis (Fig. 3) revealed potential DSP/g (based on enumeration of elongated spermatids) could be predicted by the following formula: DSP (10⁶/g) = −1.808 + (0.201 × testis weight in g) (r² = 0.65; p = 0.0001).

Study 2
There was no effect of breed on scrotal width or parameters of semen quality (p > 0.10). Mean scrotal width and seminal characteristics for the 53

Effect of Age on Spermatogenic Efficiency in 1-3 yr-old Stallions

Fig. 1. Mean (±SD) potential daily sperm production (DSP) per g of testicular parenchyma during spermatogenesis in 1–3-year-old stallions. EARLY = early primary spermatocytes; LATE = late primary spermatocytes; ROUND = round spermatids; ELONGATED = elongated spermatids. Potential DSP/g based on enumeration of elongated spermatids was significantly affected by age (means with different superscripts are different, p = 0.02).
Effect of Age on Decline in DSP/gm During Spermatogenesis in 1-3 yr-old Stallions

Fig. 2. Mean (±SD) percentage decline in potential daily sperm production (DSP) per g of testicular parenchyma during meiosis and spermiogenesis in 1–3-year-old stallions. Decline in potential DSP/g during spermiogenesis was significantly affected by age (means with different superscripts are different, p = 0.01).

Relationship of Testis Weight to Spermatogenic Efficiency in 1 to 3 yr-old Stallions

Fig. 3. Relationship between testis weight and potential daily sperm production (DSP) per g of testicular parenchyma in 1–3-year-old stallions. Potential DSP/g was calculated based on enumeration of elongated spermatids in testicular homogenates. Curved dashed lines represent 95% confidence intervals.
3-year-old stallions examined for breeding soundness are presented in Table 1. Scrotal width was not strongly correlated with total sperm number in the first (r = 0.37, p < 0.01) or second (r = 0.28, p < 0.07) ejaculates of 3-year-old stallions. The relationship between scrotal width and the number of progressively motile sperm improved from the first (r = 0.36, p < 0.01) to the second (r = 0.45, p < 0.003) ejaculates of 3-year-old stallions.

4. Discussion
As noted in previous reports, testicular weights3,4,7 and scrotal widths7–9 vary greatly in sexually immature stallions. In the present study, spermatogenic efficiency in the 1–3-year-old horses did not typically reach a value considered to be normal for adult stallions (DSP/g of 14–18 million)11 until individual testis weight reached approximately 70–80 g. Using a conversion of 1.05 g/ml of testis, combined testicular volume in horses with each testis weighing 70–80 gm should equal 133–152 ml. The majority of the 3-year-old stallions examined in Study 2 had calculated total testicular volumes greater than 152 ml, yet many would have failed to meet the criteria established by the Society for Theriogenology to be considered a “Satisfactory Breeding Prospect.” Sexually mature stallions with normal spermatogenic efficiency have been shown to produce better quality semen than sexually mature stallions with lower than normal spermatogenic efficiency.10,11 Further research will be required to determine if stallions ≤3 years of age with calculated total testicular volumes in this range have reached adult levels of spermatogenic efficiency, and if level of spermatogenic efficiency is related to semen quality in young horses.

It is noteworthy that 45% and 28% of the variation in spermatogenic efficiency in this group of 1–3-year-old stallions was explained by declines in potential yield of germ cells during meiosis and spermiogenesis, respectively. Sexually mature (≥4-yr-old) stallions with low sperm production also suffer from low yields of germ cells during spermatogenesis, particularly during meiosis and spermiogenesis.6 Cause(s) for the decline in potential yield of germ cells during spermatogenesis remain to be elucidated, so similarities in these declines during meiosis and spermiogenesis between sexually immature and mature stallions remain speculative. However, the variation in spermatogenic efficiency within similar-sized testes in the 1–3-year-old horses has been previously noted in sexually mature stallions (≥4 yr of age),10,11 so the practitioner should remain cautious in pronouncing a young stallion as having ‘normal’ testes when semen quality has not been assessed.

References